Data Evaluation Report on the Acute Toxicity of Dithane/RH-7281 80WP (10:1) to Freshwater Invertebrates – *Daphnia magna*

PMRA Submission Number {.....} EPA MRID Number 46096901

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	418959
	OECD Data Point	{.....}
	EPA MRID	46096901
	EPA Guideline	OCSPP 850.1010

Test material: Dithane/RH-7281 80WP (10:1)
Common name: Mancozeb; Zoxamide
Chemical name: IUPAC: Not Reported
CAS name: Not Reported
CAS No.: Not Reported
Synonyms: RH-117281 (zoxamide)

Purity: 7.21% (zoxamide [RH-7281])
67.8% (mancozeb [Dithane])

Primary Reviewer: John Marton, Ph.D.
Environmental Scientist, CDM Smith

Signature:
Date: 10/08/2014

Secondary Reviewer: Teri S. Myers, Ph.D.
Environmental Scientist, CDM Smith

Signature:
Date: 10/09/2014

Primary Reviewer: Freeborn G. Jewett
Biologist, EPA

Date: 10/15/2014

FREEBORN JEWETT
2014.10.17
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Secondary Reviewer(s): {.....}
{EPA/OECD/PMRA}

Date: {.....}

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	Mancozeb-014504, Zoxamide-101702	

Date Evaluation Completed: 10/15/2014

CITATION: Buchanan, J.M., B. Knight, J. Dickson. 1998. Dithane/RH-7281 80WP (10:1) Determination of Acute Toxicity (EC_{50}) to Daphnia (48h, Continuous Flow). Unpublished study performed by Inveresk Research, Tranent EH33 2NE, Scotland. Laboratory report number 16564. Study sponsored by Rohm and Haas Company, Spring House, Pennsylvania, USA. Study completed November 27, 1998.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to freshwater invertebrates. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of Dithane (mancozeb)/RH-7281 (zoxamide) 80WP (10:1) to *Daphnia magna* was studied under flow-through conditions. Daphnids were exposed to nominal RH-7281 (zoxamide) concentrations of 0 (negative control), 0.018, 0.036, 0.072, 0.144, and 0.288 mg ai/L for 48 hr. Mean-measured zoxamide concentrations were <0.004 (<LOQ; control), 0.017, 0.0335, 0.0655, 0.127, and 0.248 mg ai/L. Nominal concentrations of dithane (mancozeb) were 0 (negative control), 0.170, 0.339, 0.678, 1.356, and 2.712 mg ai/L. Mean-measured mancozeb concentrations were <0.04 (<LOQ; control), 0.217, 0.417, 0.681, 1.14, and 2.15 mg ai/L. Nominal whole product concentrations were 0 (negative control), 0.25, 0.50, 1.0,. 2.0, and 4.0 mg/L; mean-measured concentrations were 0.312, 0.601, 0.995, 1.69, and 3.20 mg/L. By test termination, mortality was 0% in the control and mean-measured 0.017 mg ai/L (zoxamide) treatment level, 93% at the mean-measured 0.0335 mg ai/L (zoxamide) treatment level, and 100% in the three highest treatment groups. The 48-hour EC_{50} was 0.025 mg ai/L based on RH-7281 (zoxamide), 0.313 mg ai/L based on dithane (mancozeb), and 0.45 mg/L based on the whole product (mancozeb + zoxamide). No sub-lethal effects were reported.

Based on the results of this study, the test material would be classified as **very highly toxic** to *Daphnia magna*, based on **RH-7281 (zoxamide)** and highly toxic based on **Dithane (Mancozeb)** concentrations, in accordance with the classification system of the U.S. EPA.

This study is scientifically sound and classified as **Supplemental** for an acute toxicity study with freshwater invertebrates; with endpoints that can be used in future risk assessments. There the mean measured concentrations of dithane (mancozeb) at two highest tested concentrations varied significantly between the 24h and 48h measurements. As a result, the endpoints were calculated without the two highest concentrations. This meant four concentrations were included in the analysis, which is less than the five concentrations that are recommended in the OPPTS 850.1010 aquatic invertebrate acute toxicity test guideline.

Results Synopsis

Test Organism Age (e.g., 1st instar): <24 hours
Test Type (Flow-through, Static, Static Renewal): flow-through

RH-7281 [Zoxamide]:
EC_{50}: 0.025 mg ai/L 95% C.I.: 0.0229-0.0272 mg ai/L
NOAEC: 0.017 mg ai/L
Probit Slope: N/A

Dithane [Mancozeb]:
EC_{50}: 0.313 mg ai/L 95% C.I.: 0.290-0.336 mg ai/L
NOAEC: 0.217 mg a.i./L
Probit Slope: N/A

Dithane [Mancozeb]/RH-7281 [Zoxamide]:
EC_{50}: 0.45 mg ai/L 95% C.I.: 0.418-0.485 mg ai/L
NOAEC: 0.312 mg ai/L
Probit Slope: N/A

Endpoint(s) Affected: Immobility

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in Series 72 of the US EPA's *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms*; US EPA Standard Evaluation Procedure, *Acute Toxicity Test for Freshwater Invertebrates*; OECD Guideline for Testing of Chemicals, 202: *Daphnia sp. Acute Immobilisation Test and Reproduction Test*; ASTM Standard E729-88a *Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians*; and the Toxic Substance Control Act No. 139 of 12/21/94 by the *Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis*- IBAMA, Brazil, when possible. The following deviations from OCSPP 850.1010 were noted:

1. Mean-measured concentrations of dithane (mancozeb) varied significantly when measured at time 0h and 48h, suggesting a solubility or analytical issue. As a result, the highest two concentrations were excluded from the analysis.
2. Pre-test health of the daphnid culture was not reported.
3. The TOC, particulate matter, chlorine, pesticide, and metal concentrations of the dilution water were not reported.
4. During the test, temperature (17.4-19.4°C) fell outside of the recommended range (20±2°C).
5. One of the test concentrations (Mean measured (MM) concentration mancozeb- 0.417 mg a.il/L; MM concentration zoxamide 0.0335 mg a.i./L; MM concentration mixture-0.601 mg a.i./L) only included 3 replications. As a result, less than the required 20 individuals were included.

These deviations impact the acceptability of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. This study was conducted in accordance with the OECD Principles of Good Laboratory Practice.

A. MATERIALS:

1. Test material Dithane (mancozeb) + RH-7281 80WP (zoxamide)

Description: yellow powder

Lot No./Batch No. : BMS-5517 (Lot No.)

Purity: 7.21% zoxamide, 67.8% mancozeb

Stability of compound under test conditions: Mean-measured concentrations of RH-7281 (zoxamide) yielded recoveries of 86 to 94% of nominal, whereas mean-measured concentrations of dithane (mancozeb) yielded recoveries of 79 to 128% of nominal.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Stored at ambient temperature in the dark.

Physicochemical properties of Dithane/RH-7281 80WP.

Parameter	Values	Comments
Water solubility at 20°C	Not Reported	
Vapor pressure	Not Reported	
UV absorption	Not Reported	
pKa	Not Reported	
Kow	Not Reported	

2. Test organism:

Species: *Daphnia magna*
(EPA preferred species is *Daphnia magna;* OECD preferred species is *Daphnia magna* or any other suitable *Daphnia* species)

Age at test initiation: 6-24 hours
(EPA recommends that Daphnids are in their first instar (#24 hrs old) and that all organisms are approximately the same size and age; OECD requires age #24 hrs old)

Source: Laboratory cultures
(EPA requires that all organisms are from the same source. Daphnids from ephippia-producing cultures should not be used; Daphnids should be from the fourth or later brood of a given parent)

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 48-hr range-finding study was conducted with nominal whole product concentrations of 0 (negative control), 0.004, 0.04, 0.4, and 4.0 mg/L. Mortality was restricted to the top dose and was 10% after 24 hours and 90% after 48 hours.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation		

Parameter	Details	Remarks *Criteria*
Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	Continuous Same as test Daphnids were fed on a diet of axenic cultures of *Selenastrum capricornutum* (Strain 278/4, CCAP, Ambleside, Cumbria). Not reported	*The recommended acclimation period is a minimum of 7 days. Organisms should not feed during the study.* *Pretest mortality should be <3% 48 hours prior to testing.*
Duration of the test	48 hours	*EPA requires 96 hours, except daphnids which are 48 hours.*
<u>Test condition</u> Static/flow-through Type of dilution system for flow-through method. Renewal rate for static renewal	 Flow-through Continuous; 5.04-5.18 vol/day N/A	*The recommended flow rates are 5 - 10 volume additions/24 hours; meter systems should be calibrated before and after the study and checked twice daily during the test period.*
Aeration, if any	None reported	
<u>Test vessel</u> Material: *(glass/stainless steel)* Size: Fill volume:	 Glass basins 3 L ~2 L	*EPA requires: small organisms in 3.9 L (1 gallon) wide mouth jars with 2-3 L of solution or daphnids and midge larvae in 250 ml jars w/ 200 ml fill*
Source of dilution water	Synthetic medium prepared	

Parameter	Details	Remarks *Criteria*
		Recommended source of dilution water is soft, reconstituted water or water from a natural, uncontaminated source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010Opdf). Dilution water should be intensely aerated before the study.

Parameter	Details	Remarks *Criteria*
<u>Water parameters</u> Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 160-166 mg/L as $CaCO_3$ 8.1-8.3 89-93% of saturation 17.4-19.4°C Not Reported Not Reported Not Reported Not Reported Not Reported	Conductivity: 0.51-0.54 mS *<u>Hardness</u>:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 140 - 250 mg/L)* *<u>pH:</u>* *EPA recommends: 7.2 - 7.6 (OECD recommends pH of 6-9); measured at start and end of test in control, high, medium, and low test concentrations* *<u>Temperature</u>:* *EPA recommends: 20°C for Daphnia (measured hourly) in at least one test vessel or if water baths are used, every 6 hr, may not vary > 1°C;* OECD *recommends* range of 18-22EC (±1EC) *<u>Dissolved oxygen</u>:* *EPA recommends: Measured at start and every 48 hours thereafter in control, high, medium, and low test concentrations.* *Static: 60-100% during 1st 48 hr and 40-100% during 2nd 48 hr* *Flow-through: 60-100% at all times*
<u>Number of replicates</u> Negative control: Solvent control: Treatments:	 4 N/A 4	One replicate in the second lowest treatment level inadvertently received no daphnids. *EPA requires 2 or more containers for each treatment group; individuals must be randomly assigned to test vessels* *OECD recommends 4 groups of 5 animals for each test concentration and the controls*

Parameter	Details	Remarks *Criteria*
<u>Number of organisms per replicate</u> Negative control: Solvent control: Treatments:	 5 N/A 5	A sixth daphnid was inadvertently added to replicate D of the top dose. *EPA/OECD requires 5 treatment levels plus one or more control groups; no more than 10% or 5% of control organisms should die during a static or flow-through study, respectively* *EPA requires a minimum of 20 daphnids in 2 or more containers per treatment; however, if a limit test is conducted, it must be shown that the LC_{50}/EC_{50} is >100 mg/L by exposing ∃ 30 organisms to ≥100 mg/L or greater. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C and # 0.5 g/L at > 17°C; flow-through: # 10 g/L at ≤ 17°C and ≤5 g/L at > 17°C.* *OECD recommends a minimum of 20 animals, preferably with 4 groups of 5 animals for each test concentration. There should be at least 2ml of test solution for each animal.*

Parameter	Details	Remarks *Criteria*
Treatment concentrations Nominal: Measured: Nominal: Measured: Nominal: Measured:	Whole Product: 0 (negative control), 0.25, 0.50, 1.0, 2.0, and 4.0 mg/L 0.312, 0.601, 0.995, 1.69, and 3.20 RH-7281 (Zoxamide): 0 (negative control), 0.018, 0.036, 0.072, 0.144, and 0.288 mg ai/L <0.004 <LOQ; control), 0.017, 0.0335, 0.0655, 0.127, and 0.248 mg ai/L Dithane (Mancozeb): 0 (negative control), 0.170, 0.339, 0.678, 1.356, and 2.712 mg ai/L <0.04 (<LOQ; control), 0.217, 0.417, 0.681, 1.14, and 2.15 mg ai/L	*Treatment concentrations should include a geometric series of at least five concentrations plus a control with each recommended concentration being at least 60% of the next higher one. The variability of measured concentrations between replicates of the same concentration should not exceed 1.5.* *OECD recommends that the highest test concentration should result in 100% immobilization and not be ≥1 g/L, while the lowest concentration should have no observable effect.*
Solvent (type, percentage, if used)	N/A; a solvent was not used	*Solvents should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-though tests. OECD recommends that the solvent not exceed 100 mg/L.*
Lighting	16L:8D (822 lux)	*EPA-recommended photoperiod is 16 hours of light and 8 hours of dark with a 15-30 minute transition period.* *OECD: optional light-dark cycle or complete darkness.*
Stability of chemical in the test system	Mean-measured concentrations of RH-7281 (Zoxamide) yielded average recoveries of 86 to 94% of nominal, whereas recoveries for dithane (mancozeb) ranged from 79 to 128% of nominal, but varied significantly between the start and termination of the experiment.	

Parameter	Details	Remarks *Criteria*
Recovery of chemical Level of Quantitation Level of Detection	 0.004 mg ai/L (RH-7281) Not Reported	
Positive control {if used, indicate the chemical and concentrations}	N/A; a positive control was not used	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks
Parameters measured including the sublethal effects	-Immobility	
Observation intervals	0, 24, and 48 hours	
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION

A. MORTALITY:

After 24 hours of exposure, immobility was 0% in the control and 0, 47, 65, 80, and 57% in the mean-measured 0.017, 0.0335, 0.0655, 0.127, and 0.248 mg ai/L (RH-7281 [zoxamide]) treatment levels. By test termination, immobility was 0% in the control and 0.017 mg ai/L treatment level, 93% in the mean-measured 0.0335 mg ai/L treatment level, and 100% in the 0.0655-0.248 mg ai/L treatment levels. The study authors reported an EC_{50} value of 0.471 mg/L based on the whole product, which corresponds to 0.0340 mg ai/L based on RH-7281 (zoxamide).

Table 3: Effect of Dithane/RH-7281 80WP (10:1) on Mortality of *Daphnia magna*

Mean-Measured and (Nominal) Zoxamide Concentrations mg ai/L	No. of Organisms	Observation Period			
		24 Hours		48 Hours	
		No Dead	% Mortality	No Dead	% Mortality
Negative Control	20	0	0	0	0
0.017 (0.018)	20	0	0	0	0
0.0335 (0.036)	15*	5	47	14	93
0.0655 (0.072)	20	13	65	20	100
0.127 (0.144)	20	16	80	20	100
0.248 (0.288)	21**	12	57	21	100
LC_{50}	0.471 mg/L (whole product); 0.0340 mg ai/L (RH-7281 [zoxamide])				
Positive control, if used Mortality: LC_{50} NOAEC:	N/A; a positive control was not used				

*One replicate did not receive any daphnids.
**One replicate received 6 daphnids.

B. SUB-LETHAL TOXICITY ENDPOINTS:

No sub-lethal effects were reported.

Table 4: Effect of Dithane/RH-7281 80WP (10:1) on Sub-lethal Effects – *Daphnia magna*

Mean-Measured and (Nominal) Zoxamide Concentrations mg ai/L	Observation Period			
	24 Hours		48 Hours	
	Endpoint	% Affected	End-point	% Affected
Negative Control	N.R.	--	N.R.	--
0.017 (0.018)	N.R.	--	N.R.	--
0.0335 (0.036)	N.R.	--	N.R.	--
0.0655 (0.072)	N.R.	--	N.R.	--
0.127 (0.144)	N.R.	--	N.R.	--
0.248 (0.288)	N.R.	--	N.R.	--
EC_{50}	Not Reported			
Positive control, if used % sublethal effect: EC_{50}	N/A; a positive control was not used			

N.R.- Not Reported

C. REPORTED STATISTICS:

The 48-hour EC_{50} value was determined using the probit analysis.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The 48-hr EC_{50} and 95% C.I. were estimated using the Untrimmed Spearman-Kärber method via CETIS statistical software version 1.8.7.12 with database backend settings implemented by EFED on 3/25/14. Analyses were conducted using the mean-measured RH-7281 (zoxamide) concentrations, mean-measured dithane (mancozeb) concentrations, and the mean-measured whole product concentrations.

RH-7281 [Zoxamide]:
EC_{50}: 0.025 mg ai/L 95% C.I.: 0.0229-0.0272 mg ai/L
Probit Slope: N/A

Dithane [Mancozeb]:
EC_{50}: 0.313 mg ai/L 95% C.I.: 0.290-0.336 mg ai/L
Probit Slope: N/A

Dithane [Mancozeb]/RH-7281 [Zoxamide]:
EC_{50}: 0.45 mg ai/L 95% C.I.: 0.418-0.485 mg ai/L
Probit Slope: N/A

E. STUDY DEFICIENCIES:

The following major guideline deficiencies led this reviewer to classify this study as **Supplemental**

1. Mean-measured concentrations of dithane (mancozeb) varied significantly between time 0h, and 48h at the two highest concentrations (nominal concentration 2 & 4 mg a.i./L), suggesting a solubility or analytical issue. The 4 mg a.i./L concentration was observed to be grey and opaque in color, and this concentration was not filtered or centrifuged. This led the reviewer to exclude the two highest concentrations from the analysis.
2. One of the test concentrations (mean measured (MM) concentration mancozeb- 0.417 mg a.il/L; MM concentration zoxamide 0.0335 mg a.i./L; MM concentration mixture-0.601 mg a.i./L) only included 3 replications. As a result, less than the required 20 individuals were included. This was the only test concentration to record partial mortality.

In addition, the following minor deficiencies were observed

1. Pre-test health of the daphnid culture was not reported.
2. The TOC, particulate matter, chlorine, pesticide, and metal concentrations of the dilution water were not reported.
3. During the test, temperature (17.4-19.4°C) fell outside of the recommended range (20±2°C).

F. REVIEWER'S COMMENTS:

The reviewer's results were based on the mean-measured RH-7281 (zoxamide), dithane (mancozeb), and the mean-measured product concentrations.

Another daphnid study submitted to The Agency evaluated the acute toxicity of a mixture of Mancozeb and Zoxamide at the same concentrations (MRID 44950502). The endpoints for the ingredients and mixture were an order of magnitude less toxic than those calculated in this study. There was no explanation for this discrepancy and nothing to indicate an issue with either study that would invalidate either set of endpoints.

Throughout the duration of the test, the test solutions at the highest treatment level were slightly opaque and very slightly gray/white in color. These test solutions were not filtered or centrifuged to characterize the nature of the suspended material. Test solutions at all other concentrations remained clear and colorless. No precipitation of test material was observed.

Experiments commenced on July 7, 1998 and were completed on August 21, 1998.

G. CONCLUSIONS:

This study is not scientifically sound and is classified as **supplemental** and **the endpoints may be used in future risk analysis**. The 48-hr EC_{50} of RH-7281 (zoxamide), dithane (mancozeb), and Dithane (mancozeb)/RH-7281 (zoxamide) 80WP was 0.025 mg zoxamide/L, 0.313 mg mancozeb/L, and 0.45 mg/L, respectively.

RH-7281 [Zoxamide]:
EC_{50}: 0.025 mg ai/L 95% C.I.: 0.0229-0.0272 mg ai/L
Probit Slope: N/A

Dithane [Mancozeb]:
EC_{50}: 0.313 mg ai/L 95% C.I.: 0.290-0.336 mg ai/L
Probit Slope: N/A

Dithane [Mancozeb]/RH-7281 [Zoxamide]:
EC_{50}: 0.45 mg ai/L 95% C.I.: 0.418-0.485 mg ai/L

Probit Slope: N/A

III. REFERENCES:

Armitage, and Berry, G. 1971. Statistical Methods in Medical Research, 2nd Ed., Blackwell Scientific Publications.

Berkson, J. 1953. Journal of American Statistical Association, 45, 565-599.

Finney, D.J. 1971. Probit Analysis, 3rd Ed., Cambridge University Press.

CETIS Summary Report

OPPTS 850.1010 Acute Invert (Daphnid) **Inveresk Research, Scotland**

Batch ID:	11-4850-9673	**Test Type:**	Mortality (48-h)	**Analyst:**	
Start Date:	08 Jul-98	**Protocol:**	OPPTS 850.1010 Acute Invert (Daphnia)	**Diluent:**	Hard Synthetic Water
Ending Date:		**Species:**	Daphnia magna	**Brine:**	Not Applicable
Duration:	NA	**Source:**	Lab In-House Culture	**Age:**	<24h

Sample ID:	14-4726-0290	**Code:**	46096901_prod	**Client:**	CDM Smith
Sample Date:	08 Jul-98	**Material:**	Dithane/RH-7281	**Project:**	
Receive Date:		**Source:**	Rohm and Haas Company		
Sample Age:	NA	**Station:**			

Batch Note: PC Code 101702 MRID 46096901; Test Material- Dithane [mancozeb]/RH-7281 [zoxamide] 80 WP (10:1)- concentrations based on whole product

Sample Note: PC Code 101702 MRID 46096901; Test Material- Dithane [mancozev]/RH-7281 [zoxamide] 80WP (10:1) concentrations based on whole product

Point Estimate Summary

Analysis ID	Endpoint	Level	mg ai/L	95% LCL	95% UCL	TU	Method
03-5470-0033	48hSurvival	EC50	0.4501	0.4177	0.485		Spearman-Kärber

48hSurvival Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	1	1	1	1	1	0	0	0.0%	0.0%
0.312		4	1	1	1	1	1	0	0	0.0%	0.0%
0.601		3	0.06667	0	0.3535	0	0.2	0.06667	0.1155	173.2%	93.33%
0.995		4	0	0	0	0	0	0	0		100.0%
1.691		4	0	0	0	0	0	0	0		100.0%
3.2		4	0	0	0	0	0	0	0		100.0%

48hSurvival Detail

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	1	1	1	1
0.312		1	1	1	1
0.601		0.2	0	0	
0.995		0	0	0	0
1.691		0	0	0	0
3.2		0	0	0	0

48hSurvival Binomials

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	5/5	5/5	5/5	5/5
0.312		5/5	5/5	5/5	5/5
0.601		1/5	0/5	0/5	
0.995		0/5	0/5	0/5	0/5
1.691		0/5	0/5	0/5	0/5
3.2		0/5	0/5	0/5	0/6

CETIS Analytical Report

OPPTS 850.1010 Acute Invert (Daphnid) — **Inveresk Research, Scotland**

Analysis ID:	03-5470-0033	**Endpoint:**	48hSurvival	**CETIS Version:**	CETISv1.8.7
Analyzed:	25 Jul-14 14:18	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes
Sample ID:	14-4726-0290	**Code:**	46096901_prod	**Client:**	CDM Smith
Sample Date:	08 Jul-98	**Material:**	Dithane/RH-7281	**Project:**	
Receive Date:		**Source:**	Rohm and Haas Company		
Sample Age:	NA	**Station:**			

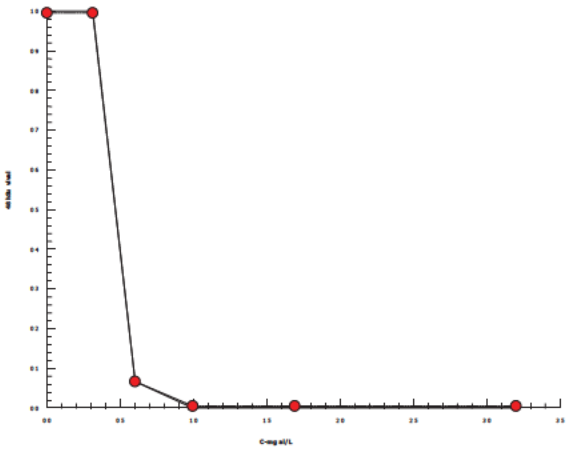
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	-0.3467	0.01622	0.4501	0.4177	0.485

48hSurvival Summary

			Calculated Variate(A/B)								
C-mg ai/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	4	1	1	1	0	0	0.0%	0.0%	20	20
0.312		4	1	1	1	0	0	0.0%	0.0%	20	20
0.601		3	0.06667	0	0.2	0.06667	0.1155	173.2%	93.33%	1	15
0.995		4	0	0	0	0	0		100.0%	0	20
1.691		4	0	0	0	0	0		100.0%	0	20
3.2		4	0	0	0	0	0		100.0%	0	21

Graphics



OPPTS 850.1010 Acute Invert (Daphnid) **Inveresk Research, Scotland**

Batch ID:	08-1794-9088	**Test Type:**	Mortality (48-h)	**Analyst:**	
Start Date:	07 Jul-98	**Protocol:**	OPPTS 850.1010 Acute Invert (Daphnia)	**Diluent:**	Hard Synthetic Water
Ending Date:		**Species:**	Daphnia magna	**Brine:**	Not Applicable
Duration:	NA	**Source:**	Lab In-House Culture	**Age:**	<24h
Sample ID:	03-0690-1807	**Code:**	46096901_zox	**Client:**	CDM Smith
Sample Date:	07 Jul-98	**Material:**	Zoxamide	**Project:**	Fungicide
Receive Date:		**Source:**	Rohm and Haas Company		
Sample Age:	NA	**Station:**			

Batch Note: PC Code 101702 MRID 46096901; Dithane [Mancozeb]/RH-7281 (Zoxamide) 80WP (10:1)- concentrations based on Zoxamide

Sample Note: PC Code 101702 MRID 46096901; Test Material- Dithane [mancozeb]/RH-7281 [zoxamide] 80WP (10:1)- concentrations based on zoxamide

Point Estimate Summary

Analysis ID	Endpoint	Level	mg ai/L	95% LCL	95% UCL	TU	Method
13-9457-9074	48hSurvival	EC50	0.02496	0.02288	0.02723		Spearman-Kärber

48hSurvival Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	1	1	1	1	1	0	0	0.0%	0.0%
0.017		4	1	1	1	1	1	0	0	0.0%	0.0%
0.0335		3	0.06667	0	0.3535	0	0.2	0.06667	0.1155	173.2%	93.33%
0.0655		4	0	0	0	0	0	0	0		100.0%
0.127		4	0	0	0	0	0	0	0		100.0%
0.248		4	0	0	0	0	0	0	0		100.0%

48hSurvival Detail

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	1	1	1	1
0.017		1	1	1	1
0.0335		0.2	0	0	
0.0655		0	0	0	0
0.127		0	0	0	0
0.248		0	0	0	0

48hSurvival Binomials

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	5/5	5/5	5/5	5/5
0.017		5/5	5/5	5/5	5/5
0.0335		1/5	0/5	0/5	
0.0655		0/5	0/5	0/5	0/5
0.127		0/5	0/5	0/5	0/5
0.248		0/5	0/5	0/5	0/6

CETIS Analytical Report

OPPTS 850.1010 Acute Invert (Daphnid) — **Inveresk Research, Scotland**

Analysis ID:	13-9457-9074	**Endpoint:**	48hSurvival	**CETIS Version:**	CETISv1.8.7
Analyzed:	25 Jul-14 14:00	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes
Sample ID:	03-0690-1807	**Code:**	46096901_zox	**Client:**	CDM Smith
Sample Date:	07 Jul-98	**Material:**	Zoxamide	**Project:**	Fungicide
Receive Date:		**Source:**	Rohm and Haas Company		
Sample Age:	NA	**Station:**			

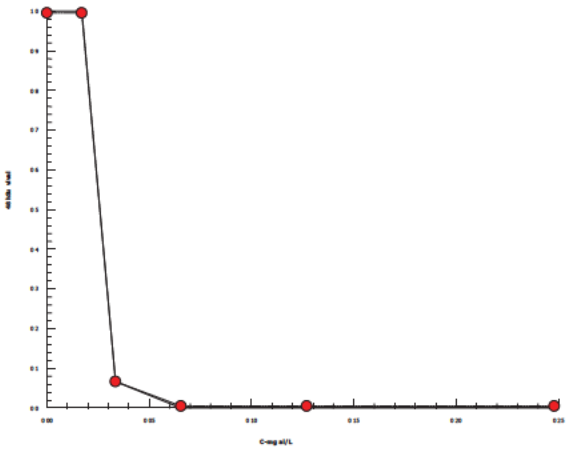
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	-1.603	0.01886	0.02496	0.02288	0.02723

48hSurvival Summary

			Calculated Variate(A/B)								
C-mg ai/L	**Control Type**	**Count**	**Mean**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**	**A**	**B**
0	Negative Control	4	1	1	1	0	0	0.0%	0.0%	20	20
0.017		4	1	1	1	0	0	0.0%	0.0%	20	20
0.0335		3	0.06667	0	0.2	0.06667	0.1155	173.2%	93.33%	1	15
0.0655		4	0	0	0	0	0		100.0%	0	20
0.127		4	0	0	0	0	0		100.0%	0	20
0.248		4	0	0	0	0	0		100.0%	0	21

Graphics



CETIS Summary Report

OPPTS 850.1010 Acute Invert (Daphnid) **Inveresk Research, Scotland**

Batch ID:	08-3666-3563	**Test Type:**	Mortality (48-h)	**Analyst:**	
Start Date:	09 Jul-98	**Protocol:**	OPPTS 850.1010 Acute Invert (Daphnia)	**Diluent:**	Hard Synthetic Water
Ending Date:		**Species:**	Daphnia magna	**Brine:**	Not Applicable
Duration:	NA	**Source:**	Lab In-House Culture	**Age:**	<24h

Sample ID:	20-9594-1157	**Code:**	46096901_manc	**Client:**	CDM Smith
Sample Date:	09 Jul-98	**Material:**	Mancozeb	**Project:**	Fungicide
Receive Date:		**Source:**	Rohm and Haas Company		
Sample Age:	NA	**Station:**			

Batch Note: PC Code 101702 MRID 46096901 Test Material: Mixture of Zoxamide and Mancozeb
Sample Note: PC Code 101702 MRID 46096901 Test Material: mixture of Zoxamide and Mancozeb

Point Estimate Summary

Analysis ID	Endpoint	Level	mg ai/L	95% LCL	95% UCL	TU	Method
21-0330-8614	48h Mortality Rate	LC50	0.3125	0.2903	0.3364		Spearman-Kärber

48h Mortality Rate Summary

C-mg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	0	0	0	0	0	0	0		
0.217		4	0	0	0	0	0	0	0		
0.417		3	0.9333	0.6465	1	0.8	1	0.06667	0.1155	12.37%	
0.681		4	1	1	1	1	1	0	0	0.0%	
1.14		4	1	1	1	1	1	0	0	0.0%	
2.15		4	1	1	1	1	1	0	0	0.0%	

48h Mortality Rate Detail

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	0	0	0	0
0.217		0	0	0	0
0.417		0.8	1	1	
0.681		1	1	1	1
1.14		1	1	1	1
2.15		1	1	1	1

48h Mortality Rate Binomials

C-mg ai/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Negative Control	0/5	0/5	0/5	0/5
0.217		0/5	0/5	0/5	0/5
0.417		4/5	5/5	5/5	
0.681		5/5	5/5	5/5	5/5
1.14		5/5	5/5	5/5	5/5
2.15		5/5	5/5	5/5	6/6

CETIS Analytical Report

Test Code: 46096901_manc | 20-9508-1898

OPPTS 850.1010 Acute Invert (Daphnid) — **Inveresk Research, Scotland**

Analysis ID:	21-0330-8614	**Endpoint:**	48h Mortality Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	17 Oct-14 9:04	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes
Sample ID:	20-9594-1157	**Code:**	46096901_manc	**Client:**	CDM Smith
Sample Date:	09 Jul-98	**Material:**	Mancozeb	**Project:**	Fungicide
Receive Date:		**Source:**	Rohm and Haas Company		
Sample Age:	NA	**Station:**			

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	-0.5051	0.01599	0.3125	0.2903	0.3364

48h Mortality Rate Summary

			Calculated Variate(A/B)								
C-mg ai/L	**Control Type**	**Count**	**Mean**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**	**A**	**B**
0	Negative Control	4	0	0	0	0	0			0	20
0.217		4	0	0	0	0	0			0	20
0.417		3	0.9333	0.8	1	0.06667	0.1155	12.37%		14	15
0.681		4	1	1	1	0	0	0.0%		20	20

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